Reprinted from
Dairy, Food and Environmental Sanitation, Vol. 14, No. 3, Pages 151-154 (March 1994)

EX. 5

Determination of Non Actionable Positives Associated with Antibiotic Tests

Stanley E. Charm, Sc.D., Charm Sciences, Inc., 36 Franklin Street, Malden, MA 02148

INTRODUCTION

The FDA has completed its evaluation of antibiotic test kits for penicillin type or beta-lactam drugs and published its results in a memorandum for state regulatory and the dairy industry, (M-I-93-3), (1). M-I-93-3 notes the minimum detection level (giving 90% positives with 95% confidence) of the various antibiotic tests for 6 target beta-lactam drugs and the FDA "Safe Levels" for these drugs. All such detection levels are equal to or less than "Safe Levels".

Of great interest is the difference between a detection level and the "Safe Level" since this was supposed to be a measure of the false violatives or non actionable positives (NAPs) associated with a test. The greater the difference the more NAPs supposedly associated with a test. This translates into the more tankers rejected that should not be rejected.

Don't confuse NAPs with false positives which are the result of test error, i.e., identifying a negative as a positive. A NAP is a true positive that is due to a drug concentration less than FDA "Safe Level"/tolerance.

It is now recognized that some detection levels noted in M-I-93-3 may not truly be the minimum detection levels, (2), but more important, the differences between detection levels and safe levels may not in fact reflect the NAPs associated with a test (3).

The FDA now plans to publish a revision of M-I-93-3 that includes the dose-response data associated with the various test kits. It is the dose-response data plotted into curves, that allows the mathematical calculation of NAPs associated with an antibiotic test. Each NAP results in a positive for a tanker and all the ramifications associated with that situation.

The major value of this calculation is the comparison of various antibiotic tests to determine which has the least number of NAPs associated.

ABOUT THE CALCULATION

If the distribution of various drug concentrations among tankers were known, it would then be possible to determine the NAPs expected in a given time with a particular test. This information is not known. However, over varying periods of time every drug concentration will be present in a number of tankers, for example in 100 tankers. The time it takes for this to occur will be less for lower concentrations. It will depend on the frequency of each drug used, how they are used, and their withdrawal times. Over some period of time there will have been 100 tankers with each concentration appearing at the plant and tested. In essence, this is basing the calculation on a uniform distribution of concentration among tankers for the 6 target beta-lactam drugs ranging from zero to the drug "Safe Level", (Pen G, ceftiofur, cephapirin, cloxacillin, ampicillin, amoxicillin).

The dose-response data used in these calculations are published data for the test kits, (4), but the tests are not named.

DOSE-RESPONSE CURVES AND CALCULATION OF NAPs AND NAPC

In Table 1 are presented dose-response data for pen G for 3 different tests A, B, C. The data plotted as dose-response curves are shown in Figures 1 and 2, where Figure 1 compares test A with test C and Figure 2 compares test B with test C.

Table 1. Dose-response Data for three Penicillin G tests, A, B, and C.

TEST A		TEST B		TEST C	
Conc. ppb	%pos	Conc. ppb	%pos	Conc. ppb	%pos
0	0	0	0	0	0
1	17	1	0	1	0
2	38	2	0	2	0
3	100	3	100	2.7	0
4	87	4	100	3.5	50
5	100	5	100	4	100
6	100	6	100	4.8	100
				6.0	100

The dose-response curve for the pen G test giving zero NAPs is shown in Figure 3 along with the dose-response for the test giving the maximum NAPs. The area under the dose-response curve represents a concentration. In Figure 3, fraction positive is noted on the "y" axis. A fraction positive of 1 multiplied by 5ppb on "x" axis gives an area that represents 5ppb. This is the concentration associated with the maximum NAPs or the NAP concentration, NAPC.

Reversing the procedure, dividing the area by the "Safe Level", the fraction positive associated with the test for the drug is found. In Figure 3, area 5ppb divided by "Safe Level"

Figure 1.

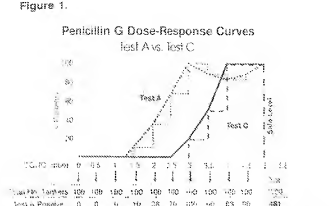


Figure 2.

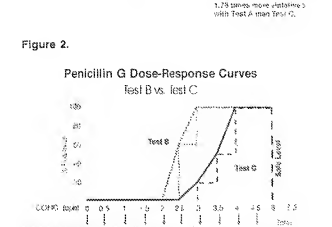
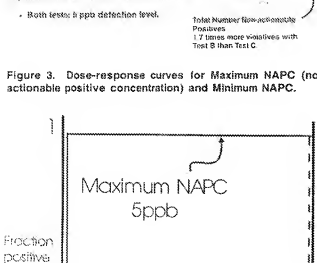


Figure 3. Dose-response curves for Maximum NAPC (non-actionable positive concentration) and Minimum NAPC.

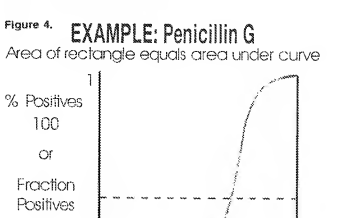


5ppb is 1 or × 100 = 100%. All tests for pen G will have NAPC between zero and 5ppb.

Although the data presented for dose-response curves are not ideal since there are only 6 replicates associated with each point, it is possible to make useful estimates. Some curves may show "dips" and "peaks" rather than a smooth curve and this is a symptom of erratic results. Since it is known that the shape of the dose-response curve must be sigmoid, (Figure 4), it is possible to statistically improve the plotted data by submitting it to a probit analysis. However, in this calculation take the actual curves realizing that "dips" and "peaks" do not occur with a large number of samples, and should be smoothed out to look like Figure 4.

In Figure 4, it is shown how area under curve divided by "Safe Level" concentration gives the fraction positive of NAPS associated with the test.

Figure 4.



COMPARISON OF NAPs ASSOCIATED WITH TESTS A, B, AND C

Referring to Figure 1, for pen G assume 100 tankers eventually come to the plant containing concentrations between 0 and 5ppb and all are tested by tests A & C. Starting at zero and moving along the concentration, ("x" axis), the number of tankers positive for each concentration using a .5ppb interval is found. For test A this is 481, and test C 270 out of the 1000 tankers tested with each test.

Thus. test A has 481/1000 × 100 = 48.1% NAPs
and test C = 270/1000 × 100 = 27%.

A smaller concentration interval, e.g., .2ppb would result in a more accurate determination.

Using the method of calculating NAPs from area under curve, (or from the NAPC), the squares under each curve are counted. For example, there are 15 squares under curve C and 25 squares under curve A. Converting % positive to fraction positive by dividing by 100, each square in Figure 1 is equivalent to .2 × .5 = .1ppb. Therefore, the NAPC associated with test A is 25 × .1 = 2.5ppb and with test C 15 × .1 = 1.5ppb. Calculating the fraction positive NAPs by

dividing NAPC with "Safe Level": for test A, 2.5/5 = .5 or 50% and for test C, 1.5/5 = .3 or 30%.

In comparison test A has 48.1/27 = 1.78 or 78% more NAPs than test C if calculated from number of tankers and 50/30 = 1.66 or 66% if calculated from NAPC.

If calculated from a probit analysis with a mathematical integration, test A has 92% more NAPs than test C. The probit analysis statistically smoothed out the "dip" in the test A curve giving a greater area than determined with the "dip". This is the most accurate determination.

Referring to Figure 2 and similarly comparing test B with test C, test B has 70% more NAPs than test C counting tankers.

DIFFERENCES BETWEEN DETECTION LEVELS AND SAFE LEVELS DO NOT CORRELATE WITH NAPs ASSOCIATED WITH TESTS

In M-I-93-3, these 3 tests have essentially the same detection levels noted for pen. G. This proves detection level-Safe Level difference does not determine expected NAPs.

DETERMINING THE TEST WITH LEAST NAPs

To determine the test with the smallest number of NAPs this calculation should be carried out for each of the 6 target beta-lactam drugs.

For a given test, the NAPs for each drug are added and compared with other similar tests. The test with the smallest number will have the least number of non actionable positives associated with it, e.g., see equation (1).

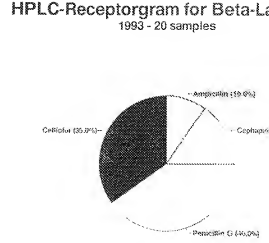
(1) Total non actionable positives associated with test = (NAPs) pen G + (NAPs) clox + (NAPs) ceft + (NAPs) amox + (NAPs) ampi + (NAPs) ceph

Some tests don't detect all six target drugs. Such tests will have false negatives for these undetected drugs. Thus, be sure to compare tests that detect the same drugs.

THE DRUG INCIDENCE FACTOR

The frequency of use of a drug also influences equation (1). For example, if pen G is more commonly used than cloxacillin the (NAPs) pen G could be weighted with an incidence factor so that the total NAPs are influenced more by pen G than by cloxacillin. With the HPLC-Receptorgram our identification lab has identified 20 positive beta-lactams sent to it in 1993 with distribution as shown in Figure 5. Pen G was found 40% and ceftiofur 35% as the two major beta-lactam drugs. If drug incidence is available, the NAPs for each drug in equation (1) could be multiplied by its drug incidence to give a more refined total. However, the data in Figure 5 has not been substantiated as representative. In view of this, the incidence of each drug may be considered equal and equation (1) used as is.

Figure 5.

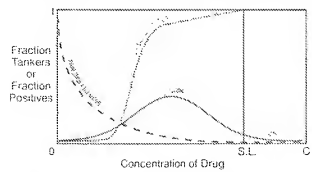


A UNIFIED SOLUTION FOR CALCULATING EXPECTED POSITIVES, (Actionable and Non-actionable) FOR VARIOUS DRUGS WITH VARIOUS TESTS

It was noted previously that to determine the actual number of NAPs or APs (actionable positives) expected at a plant, the drug concentration distributed among tankers must be known. In Figure 6, a distribution curve is shown assuming that more tankers have lower concentrations (see fraction tankers vs. concentration).

Figure 6.

A Unified Solution for Calculating Expected Positives, (Actionable and Non-actionable) for Various Drugs with Various Tests



By multiplying the test dose-response curve by the fraction tankers-concentration curve the product is the NAPs-APs curve.

The area under this curve is the total number of positives. The Safe Level (S.L.) is a concentration selected by FDA that divides total positives into NAPs and APs.

The mathematical notation indicating what has been described is shown in equations 2 and 3. In equation 4 is shown the effect of not using a tanker-concentration curve. This in effect simply assumes a uniform concentration distribution among tankers. In comparing dose-response

curves and NAPs with different tests, but for the same drug, the constant K representing the uniform distribution cancels out.

Fraction positives = fraction positive for test, (dose-response)
or
= fraction positive tankers NAPs or APS. (NAPS are non-actionable positives, APS are actionable positives)

C = any drug concentration

S.L. = Safe Level concentration

Fraction tankers = fraction of tankers having various concentrations of a drug

Fraction tankers as a function of concentration = F^I (C)

Test fraction positives for drug as a function of concentration (dose-response) = F^{II} (C)

Equations for fraction positive tankers associated with the test

$$= \int_0^{S.L.} \frac{F^I(C) \cdot F^{II}(C)\, dc}{S.L.} \quad \text{(2) Non-actionable}$$

$$+$$

$$= \int_{S.L.}^{C} \frac{F^I(C) \cdot F^{II}(C)\, dc}{C\text{-}S.L.} \quad \text{(3) Actionable}$$

Without knowing F^I (C), it is assumed that drugs are uniformly distributed among tankers, (i.e. a straight line in place of curve). This means F^I (C) = constant (e.g. K) and

$$\text{fraction NAPs} = K \int_0^{S.L.} \frac{F^{II}(C)\, dc}{S.L.} \qquad (4)$$

CONCLUSION

Differences between FDA "Safe Levels" and detection levels do not measure number of NAPs associated with antibiotic tests. The area under the dose-response curve for the test is related to the NAPs. By adding the NAPs associated with each drug, the test giving the smallest number will be the one with the least NAPs.

REFERENCES

(1) FDA, CFSAN memorandum, M-I-93-3, November 26, 1993.
(2) FDA statement made at Laboratory Committee Meeting, January 10, 1994, St. Louis, MO.
(3) Charm, S.E., 1994. "Why Differences Between Detection Levels and Safe Levels Do Not Measure False Violatives Associated With a Test." Talk delivered at FDA-CVM, January 7, 1994.
(4) FDA approved test kit inserts or revised M-I-93-3 (in press).